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Garlic extract induces intestinal P-glycoprotein, but exhibits no effect on intestinal and hepatic CYP3A4 in humans

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Abstract

Garlic extracts have been shown to decrease drug exposure for saquinavir, a P-glycoprotein and cytochrome P450 3A4 substrate. In order to explore the underlying mechanisms and to study the effects of garlic on pre-systemic drug elimination, healthy volunteers were administered garlic extract for 21 days. Prior to and at the end of this period, expression of duodenal P-glycoprotein and cytochrome P450 3A4 protein were assayed and normalized to villin, while hepatic cytochrome P450 3A4 function and simvastatin, pravastatin and saquinavir pharmacokinetics were also evaluated. Ingestion of garlic extract increased expression of duodenal P-glycoprotein to 131% (95%CI, 105-163%), without increasing the expression of cytochrome P450 3A4 which amounted to 87% (95%CI, 67-112%), relative to baseline in both cases. For the erythromycin breath test performed, the average result was 96% (95%CI, 83-112%). Ingestion of garlic extract had no effect on drug and metabolite AUCs following a single dose of simvastatin or pravastatin, although the average area under the plasma concentration curve (AUC) of saquinavir decreased to 85% (95%CI, 66-109%), and changes in intestinal P-glycoprotein expression negatively correlated with this change. In conclusion, garlic extract induces intestinal expression of P-glycoprotein independent of cytochrome P450 3A4 in human intestine and liver.

Key words: herbal medicines, drug interactions, MDR1, P-glycoprotein, pravastatin, simvastatin, saquinavir.
1. Introduction

Use of over-the-counter herbal medicines is increasing, while also becoming more common, with one out of five patients currently using natural products (Navarro, 2009, King et al., 2009). However, data on the efficacy and safety of herbal medicines are often incomplete (O'hara et al., 1998, Bent, 2008). Furthermore, drug interactions between herbal medicines and prescription or over-the-counter medications can be hazardous in some cases (Izzo and Ernst, 2009). For example, St John’s Wort has been shown to decrease the efficacy of several drugs, including oral contraceptives (Schwarz et al., 2003) and HIV protease inhibitors (Piscitelli et al., 2000). In these cases, the induction of several cytochrome P450 drug metabolizing enzymes and the intestinal efflux transporter P-glycoprotein (PGP) have been detected (Dürr et al., 2000).

Both experimental data, and data from controlled clinical trials, suggest that garlic preparations may lower lipid levels and have anti-hypertensive and anti-coagulant properties, which could lower cardiovascular risk factors for patients (Stevinson et al., 2000, Ried et al., 2008, Rahman, 2007, O'hara et al., 1998). The efficacy of garlic and its derivatives have been linked to the generation of organosulfurs, such as monosulfides, polysulfides, and ajoenes, by the body in response to the alliin contained in garlic (Iciek et al., 2009, Rose et al., 2005). Therefore, alliin content is considered to be a critical component of garlic preparations.

Currently, there are three studies that have investigated the effects of garlic on CYP450 drug metabolizing enzymes in humans (Gurley et al., 2002, Markowitz et al., 2003, Gurley et al., 2005). Using debrisoquine, midazolam, caffeine, and chlozoaxazone as test substrates, Gurley et al. showed that garlic oil decreases CYP2E1 activity by 39% and 22% in young and elderly volunteers, respectively, yet exhibits no effect on CYP1A2, 2D6, and 3A4 function (Gurley et al., 2002, Gurley et al., 2005). Similarly, Markowitz et al. confirmed that garlic exhibits no effect on CYP2D6 and CYP3A4 function in drug interaction studies of Kwai garlic.
supplements from Lichtwer Pharma and dextromethorphan or alprozolam (Markowitz et al., 2003). In contrast, results from a clinical trial of 9 healthy volunteers demonstrated that 600 mg of garlic extract from GarliPure® twice a day (b.i.d.) administered for 21 days decreased the average area under the plasma concentration curve (AUC) and the average maximal plasma concentration ($C_{\text{max}}$) of saquinavir by 51% and 54%, respectively (Piscitelli et al., 2002). The protease inhibitor, saquinavir, is a substrate of CYP3A4 and PGP (Plosker and Scott, 2003), suggesting that induction of CYP3A4 and/or PGP by garlic extracts may represent the underlying mechanism.

To further examine the effects of garlic on pre-systemic drug elimination, healthy volunteers were administered garlic extract (GarliPure®) b.i.d. over 21 days. Levels of PGP, also known as MDR1, and CYP3A4 in duodenal biopsy samples were then evaluated, and hepatic CYP3A4 function was assessed using the erythromycin breath test (EMBRT). Due to overlapping treatment indications, the probability of concurrent intake of garlic extracts and statins is high. Therefore, the effects of garlic extract on the pharmacokinetics of a single dose of simvastatin or pravastatin, i.e. a CYP3A4-dependent and -independent metabolized statin, respectively, were evaluated. Furthermore, a pharmacokinetic study of a single dose of saquinavir was performed to directly compare these results with those from a previous study by Piscitelli et al. (Piscitelli et al., 2002).
2. Methods

2.1. Materials

Garlic extract (GarliPure®, 600 mg caplets from Natrol®, CA, USA containing gamma-glutamylcysteines 12’000 µg, 4’800 µg alliin, 3’900 µg sulfur and 3’800 µg thiosulfimates resulting in a allicin yield 3’600 µg), simvastatin (Zocor®, 20mg), pravastatin (Selipran®, 20 mg), and saquinavir (Fortovase®, 200 mg) were purchased from their respective manufacturers. 14C-erythromycin breath test kits were obtained from Metabolic Solutions Inc. (Nashua, NH, USA).

2.2. Clinical study

This study protocol was approved by the ethics committee of the Canton of Zürich and the Swiss Federal Office of Health (BAG). An open-label, randomized, cross-over design was used with two study periods. Ten non-smoking, male Caucasian subjects were enrolled that ranged in age from 24-38 y (mean 28.8 y), in body weight from 54.0-92.0 kg (mean 74.4 kg), and in body mass index (BMI) from 19.8-25.5 kg*m⁻² (mean 22.5 kg*m⁻²). Based on medical histories, physical examinations, standard clinical chemistry and hematology analyses, all subjects were considered healthy. The subjects were instructed to abstain from taking any type of medication, including over-the-counter remedies and supplements, grapefruit, caffeine, or alcohol-containing food or beverages for at least 3 d prior to the start of the study and throughout the course of the study. Written informed consent was obtained from each subject prior to participation.

Each study period consisted of 4 d. On the first morning, volunteers arrived at 7:30 AM after fasting overnight. Blood samples were collected prior to the drug administration (8:00 AM) and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h thereafter. On the first and second days, volunteers randomly received either a single dose (20 mg) of simvastatin or pravastatin, followed by
standardized meals served 4 and 10 h after dosing. On the third day, volunteers received breakfast (consisting of 300 ml unskimmed milk, 2 small bread rolls, 10 g of butter, 4 thin slices of salami, 1 boiled egg and 25 g of corn flakes and contained 30 g of fat, 83 g of carbohydrates and 25 g of proteins) and two hours later a single dose (1200 mg) of saquinavir. Blood samples were collected just before saquinavir was administered, as well as 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after receiving saquinavir. Additional standardized meals were served 2 and 8 h after dosing.

All blood samples were immediately centrifuged following collection at 2500 rpm and 4°C for 10 min, then stored at –20°C. On the fourth day, volunteers underwent a gastroduodenoscopy under midazolam sedation. Seven biopsy samples of duodenal intestinal mucosa were obtained. One biopsy was used for histopathologic examination, while the others were immediately frozen in liquid nitrogen and stored at –70°C. In the afternoon of the same day, an EMBRT was performed according to the manufacturer’s instructions. Thereafter, all subjects received a garlic extract dose of 600 mg twice daily for 21 days. On days 19-22, the same procedures described above for days 1-4 were repeated.

2.3. Pharmacokinetics

Plasma concentrations of simvastatin, simvastatin acid, pravastatin, pravastatin lactone, R-416, and saquinavir were determined using liquid chromatography-mass spectrometry (LC-MS) as described before (27). Except for saquinavir, the quantification limit was 0.01 µg/l, between-day precision was < 10%, and the accuracy of detection ranged from 84.5 – 112%. The corresponding values for saquinavir were 0.05 mg/l as quantification limit, < 6.2% for between-day precision and a range of 99 – 100% for accuracy. Areas under the plasma concentration-time curves up until the last quantifiable concentration (AUC_{0-n}) were calculated using the trapezoidal rule.
The sample size was determined based on the estimates reported in a interaction study on the effects of St. John’s wort on simvastatin exposure (Sugimoto et al., 2001). Based on an expected mean difference for total AUC of 15.2 ng h/ml and a standard deviation of AUC change of 14.9 ng h/ml, a sample size of 10 ($\alpha=0.05$, 2-tailed) results in a power of 0.81.

2.4. Western blot analysis

Frozen biopsy specimens were thawed and processed exactly as described in (8). Protein concentrations of the homogenates were determined using the bicinchoninic assay protein determination kit from Pierce (Rockford, IL, USA). SDS-polyacrylamide gel electrophoresis and Western blotting are described in (8, 28) and were performed with the following monoclonal antibodies: C219 for the detection of MDR1 (Alexis, Lausen, Switzerland), CYP3A4 (Gentest, Woburn, MA, USA) and villin (Chemicon, Temeluca, CA, USA). Blots were developed with the Uptilight detection kit from Interchim, Montfuçon, France). After development, films were scanned with a CAMAG TLC Scanner II (CAMAG, Muttenz, Switzerland) for comparison of relative protein expression levels and normalization to villin.

2.5. Statistics

The geometric means of CYP3A4 and MDR1 ratios to villin protein were calculated from 3 individual biopsy specimens, and AUC$_{0-n}$ data and ERMBT results were compared before and after treatment with garlic using the paired Wilcoxon signed-rank test. All calculations and drawings were done in Microsoft Excel and (S)-Plus 6.1 for Windows (Insightful Corporation, 2002).
3. Results

3.1. Intestinal expression of P-glycoprotein/MDR1 and CYP3A4 protein and CYP3A4 function in liver

Ten male, healthy volunteers consumed garlic extract (2 doses of 600 mg per day) for 3 weeks, and intestinal biopsy specimens were collected before and after the garlic intake period. PGP, CYP3A4, and villin were detected by western blot, and a representative analysis is shown in Figure 1. For each volunteer, protein levels before and after garlic intake were compared, and an increase in PGP expression can already be visually detected in 6 of the volunteers (subjects B, C, E, F, H, and I). In contrast, paired samples for subjects A, D, and G were difficult to compare due to differences in epithelial content (based on detection of villin expression). Expression of CYP3A4 was largely unaffected by ingestion of garlic extract. Based on densitometric analysis of western blot detection, and normalization to levels of villin, 3 pairs of biopsy specimens collected before and after garlic intake were averaged for each individual using geometric means (Fig. 2, left panel). Intestinal PGP protein levels were found to increase after intake of garlic extract in 9 out of the 10 volunteers assayed. When the PGP/villin ratio detected prior to garlic intake was set to 100%, the average PGP/villin content after receiving garlic extract was 131%, with a 95% confidence interval (95% CI) ranging from 105-163% ($p = 0.0195$). Similarly, CYP3A4 levels prior to and following intake of garlic extract intake were normalized to villin, and the average CYP3A4/villin content after garlic intake was 87%, with a 95% CI ranging from 67-112%. Ingestion of garlic extract also exhibited no effect on the EMBRTs performed (Fig. 2), with the geometric mean of EMBRTs performed before and after garlic intake having a ratio of 96%, with a 95% CI ranging from 83-112%. Thus, these data demonstrate a consistent and significant increase in intestinal PGP protein in response to garlic extract, yet no difference in levels of CYP3A4 in the intestine and CYP3A4 function in the liver.
3.2 Simvastatin kinetics

When average simvastatin plasma concentration time profiles were compared before and at the end of 3 weeks of garlic extract intake, they were slightly higher after the ingestion of garlic extract (Fig. 3, left panel). Accordingly, the mean AUC after garlic intake was 22.1 ± 25.7 µg h/l, compared to 11.2 ± 5.7 µg h/l at baseline. Large increases in the simvastatin plasma concentrations after garlic extract intake for subjects A and F were largely responsible for the overall increase in simvastatin levels detected (Fig. 3), resulting in a geometric mean AUC ratio of 137%, with a 95% CI ranging from 88-212%. For the metabolite, simvastatinic acid, its average plasma concentration time profile, AUC, and other pharmacokinetic parameters were similar before and after garlic extract intake (Fig. 3, Table 1). Hence, no significant changes, in particular no decrease in the bioavailability of simvastatin, was detected following garlic extract intake.

3.3 Pravastatin kinetics

Average plasma concentration time profiles for pravastatin, pravastatin lactone, and the active metabolite, R-146, were similar before and at the end of the ingestion of garlic extract (Fig. 4). Also AUCs (Fig. 4) and pharmacokinetic parameters (Table 1) did not show any effects from the ingestion of garlic extract. Accordingly, the geometric mean AUC ratios for pravastatin, pravastatin lactone, and R-146 were 94% (95% CI, 65-136%), 84% (95% CI, 40-174%), and 89% (95% CI, 68-115%), respectively. These results did not indicate any clinically relevant effects from garlic intake on pravastatin pharmacokinetics.
3.4. Saquinavir kinetics

When saquinavir concentrations were assayed following ingestion of garlic extract at 3 h and 4 h time points, the average saquinavir concentration was found to be lower compared to baseline (Fig. 5, left panel). Furthermore, the ratio of AUC from 0 to 8 h following garlic extract intake were found to decrease in 7 of the study subjects (A-C, E, H-J), and increase in the other 3 subjects (D, F, G) (Fig. 5). Overall, the geometric mean AUC ratio decreased to 85% from baseline, with a 95% CI ranging from 66-109%. In contrast, the corresponding values for terminal half-life slightly increased to 120%, with a 95% CI ranging from 94-152%. Since PGP has been hypothesized to limit saquinavir bioavailability, and ingestion of garlic extract was found to increase intestinal PGP in this study, linear regression analysis of individual changes in saquinavir AUC, i.e. the corresponding AUC ratios before and after garlic intake, was performed to explore the effects of changes in intestinal PGP content (Fig. 5). A negative correlation was identified, with an $R^2$ of 0.467 ($p = 0.029$).
4. Discussion

This is the first study, to our knowledge, that demonstrates that garlic extract intake can increase intestinal expression of PGP. This result is consistent with the reduced bioavailability of saquinavir previously observed following ingestion of garlic extract (Piscitelli et al., 2002). This study also demonstrates that garlic extract exhibits no effect on intestinal expression of 3A4, does not change hepatic 3A4 function, and does not reduce the bioavailability of simvastatin. These results are consistent with a previous report that showed no changes in the pharmacokinetics of the CYP3A4 substrates, midazolam and alprazolam (Markowitz et al., 2003, Gurley et al., 2005, Gurley et al., 2002). Therefore, our results demonstrate a differential induction of PGP and CYP3A4 in response to garlic extract, suggesting that garlic extract can induce intestinal PGP independent of effecting CYP3A4.

After 3 weeks of garlic extract intake, expression of intestinal PGP increased 31%, which is consistent with the 37% increase detected for intestinal PGP after 2 weeks of ingesting St. John’s Wort by 8 healthy volunteers (8), with the same western blotting procedures used (Dürr et al., 2000). In previous studies of St John’s Wort digoxin interactions, increased expression of PGP was associated with an 18-25% decrease in digoxin AUC (Johne et al., 1999, Dürr et al., 2000). The decrease in the saquinavir AUC observed in this study was slightly less at 15%, with a rather broad 95% CI of (-9)-34%. We hypothesize that this considerably larger variability associated with saquinavir versus digoxin is due to the low bioavailability of saquinavir (~12% for the soft gelatin capsules), and its food-dependent absorption properties (Plosker and Scott, 2003). To facilitate the drug absorption of saquinavir in this study, administration of the soft gelatin capsules was combined with breakfast. However, the variability observed with these studies was still too great to achieve statistical significance, even with a treatment group of 10 volunteers. Although, the data do show a clear and significant linear negative correlation between change in intestinal PGP expression and
changes in the bioavailability of saquinavir following ingestion of garlic extract. The intestinal PGP ratio calculated also explains the 46.7% variability associated with the saquinavir AUC ratio, and thus confirms the results of previous studies that demonstrated that the intestinal efflux transporter, PGP, limits the oral bioavailability of saquinavir (Plosker and Scott, 2003).

A study by Piscitelli et al. reported that 3 weeks of garlic extract intake reduced the AUC for orally administered saquinavir to 49.5% of pretreatment values with a 90% CI ranging from 30.8-79.3% (Piscitelli et al., 2002). In contrast, the AUC for saquinavir following 3 weeks of garlic extract intake in this study was slightly less pronounced at 85%, with a 95% CI ranging from 66-109%. In order to directly compare these two studies, the same garlic extract preparation, dosage, saquinavir preparation, and treatment period were maintained in this study. Several drugs with rather short half-lives were selected in order to allow single dose pharmacokinetics to be evaluated. Therefore, a 3-day course of saquinavir in combination with garlic extract was not performed. Mechanistically, we hypothesize that this preference in the study design does not account for differences in the magnitude of the effects observed, but rather, the observed differences are the result of large intersubject and intrasubject variabilities in saquinavir absorption. Pravastatin is a well-characterized substrate of the hepatic organic anion transporter, OATP1B1 (Ito et al., 2005). The absence of any observed effects on pravastatin pharmacokinetics following 3 weeks of garlic extract intake, suggests that the Garlipure® form of garlic extract does not significantly influence the expression and function of OATP1B1 in humans.

We used the EMBRT to test for changes in hepatic CYP3A4 function. However, it has been previously reported that erythromycin is also a PGP substrate and that the concurrent administration of a PGP inhibitor may increase hepatic erythromycin metabolism and result in false positive EMBRTs, i.e. increases in the EMBRT without CYP3A4 induction (Frassetto et
al., 2007, Kurnik et al., 2006). In our study, the focus was on PGP and 3A4 induction and no change in EMBRT was observed. Since also the clearance of the CYP3A4 substrate simvastatin was not increased after garlic extract intake, we are confident about the conclusion that garlic extracts do not induce CYP3A4 in the liver is clinically valid despite of the possible limitations of the EMBRT.

The nuclear receptors, pregnane X receptor (PXR) and constitutive androstane receptor (CAR), have been shown to regulate the expression of drug transporters and metabolizing enzymes in liver and intestine, including CYP3A4 and MDR1 (Kohle and Bock, 2009, Di Masi et al., 2009). The hyperforin contained in St. John’s Wort has been shown to be a strong ligand of human PXR, with PXR-mediated induction considered to be the underlying mechanism for the coordinated induction of CYP3A4 and MDR1 by St. John’s Wort (Tirona and Bailey, 2006). Studies in rat further suggest that the diallyl sulfide contained in garlic is also a structurally atypical phenobarbital-type inducer (Dragnev et al., 1995), and that diallyl sulfide acts as an activator of rat CAR (Chang, 2009). Accordingly, activation of CAR by diallyl sulfide might be responsible for the observed induction of MDR1 by garlic extracts. However, it has also been suggested that CAR controls the expression of CYP3A4 as well (Kohle and Bock, 2009, Di Masi et al., 2009). However, diallyl sulfide is mainly found in garlic oils, but only minor amounts of diallyl sulfide have been found in garlic powder tablets (Lawson et al., 1991). Therefore, it remains to be investigated why garlic increases MDR1 in intestine, but does not similarly effect CYP3A4 in intestine and liver.
In contrast with the results of this study, previous studies in rats have demonstrated that garlic oil and its constituents, diallyl sulfide, diallyl disulfide, and diallyl trisulfide, increase mRNA and protein levels of CYP3A1 and other CYP proteins (Wu et al., 2002, Dragnev et al., 1995). In combination, these results suggest that there are interspecies differences in the regulation of CYP3A orthologues between rodents and humans.

In conclusion, this study demonstrates a differential induction of PGP versus CYP3A4 in human intestinal samples from healthy volunteers, where garlic extract increased intestinal expression of PGP independent of CYP3A4. Based on these results, decreased bioavailability of the PGP substrate, saquinavir, would be predicted in the presence of garlic extract. In addition, garlic extract did not exhibit any effects on hepatic CYP3A4 and OATP1B1 function, and exhibited no pharmacokinetic interaction with simvastatin and pravastatin. Therefore, garlic extract represents an herbal medicine that can be safely combined with statins for the treatment of hypercholesterolemia.
Acknowledgements

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Conflict of Interest / Disclosure

The authors declare no conflict of interest.
References


Chang, T. K., 2009. Activation of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) by herbal medicines. AAPS J, 11, 590-601.


Figure Legends

**Figure 1:** Expression of intestinal P-glycoprotein and CYP3A4 protein before and after ingestion of garlic extract for three weeks. Ten healthy male volunteers ingested garlic extract for 3 weeks, with duodenal biopsy specimens collected before and at the end of the study period. Homogenates (100 μg) of individual biopsy specimens were incubated with antibodies against either P-glycoprotein or CYP3A4, visualized by chemiluminescence. Membranes were then stripped and probed with antibodies against villin. A representative blot is shown from the 3 sets of biopsy specimens analyzed.

**Figure 2:** Ratios of P-glycoprotein/MDR1 and CYP3A4 to villin, and EBMRTs performed. Intestinal P-glycoprotein (left panel) and CYP3A4/villin (middle panel) expression ratios for subjects A-K were determined using densitometric analysis of western blots and are given as the geometric means of 3 individual duodenal specimens obtained before and after garlic intake. EMBRTs (right panel) were performed before treatment (control) and after treatment (garlic). In each panel, the bold black line corresponds to the geometric mean value before and after garlic intake, whereas the thin gray lines give the individual values for subject A to K.

**Figure 3:** Effects of garlic extract ingestion on simvastatin pharmacokinetics. Left panel: Plasma concentration-time profiles (mean ± SEM) for subjects A-K for simvastatin (triangles) and simvastatin acid (circles) before (open symbols) and after 3 weeks of garlic extract intake (filled symbols). Right panel: Comparison of simvastatin AUC profiles for the after administration of simvastatin or simvastatin acid alone (controls in each case) versus each in combination with garlic intake for subjects A-K. The bold black line corresponds to the
geometric mean values, whereas the thin gray lines give the individual values for subject A to K.

**Figure 4:** Effects of garlic extract ingestion on pravastatin pharmacokinetics. Left panel: Plasma concentration-time profiles (mean ± SEM) for subjects A-K for pravastatin (triangles), pravastatin lactone (circles), and R-416 (insert), before (open symbols) and after 3 weeks of garlic extract intake (filled symbols). Right panels: Comparison of AUC ratios for pravastatin, pravastatin lactone, and R-416 before and after garlic intake for subjects A-K. The bold black line corresponds to the geometric mean values, whereas the thin gray lines give the individual values for subject A to K.

**Figure 5:** Effects of garlic extract ingestion on saquinavir pharmacokinetics. Left panel: Saquinavir plasma concentration-time profiles (mean ± SEM) for subjects A-K before (open symbols) and after 3 weeks of garlic extract intake (filled symbols). Middle panel: Comparison of saquinavir AUC ratios before and after garlic intake for subjects A-K. The bold black line corresponds to the geometric mean values, whereas the thin gray lines give the individual values for subject A to K. Right panel: Correlation of changes in saquinavir AUCs and intestinal PGP expression, with data points representing the individual values for subjects A-K, with the best-fit line generated by linear regression analysis ($R^2 = 0.467, p = 0.029$).
Table 1: Simvastatin and pravastatin kinetics before and after garlic extract intake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Garlic</th>
<th>Geometric mean ratio (95% CI)</th>
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<tbody>
<tr>
<td><strong>Simvastatin</strong></td>
<td></td>
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<tr>
<td>AUC0-24h (ug . h/L)</td>
<td>11.2 ± 5.7</td>
<td>22.1 ± 25.7</td>
<td>1.37 (0.88, 2.12)</td>
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<tr>
<td>CL/F (L/h)</td>
<td>2349 ± 1340</td>
<td>2146 ± 1660</td>
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<tr>
<td>t½</td>
<td>4.92 ± 0.93</td>
<td>5.00 ± 1.90</td>
<td>1.02 (0.81, 1.27)</td>
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<tr>
<td>tmax (h)</td>
<td>1.4 ± 0.7</td>
<td>1.5 ± 0.9</td>
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<tr>
<td>Cmax (ug/L)</td>
<td>3.85 ± 1.99</td>
<td>5.25 ± 6.32</td>
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<tr>
<td><strong>Simvastatin acid</strong></td>
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<tr>
<td>AUC0-24h</td>
<td>7.1 ± 3.2</td>
<td>9.1 ± 5.2</td>
<td>1.25 (0.80, 1.93)</td>
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<td><strong>Pravastatin</strong></td>
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<tr>
<td>AUC0-24h (ug . h/L)</td>
<td>41.8 ± 24.1</td>
<td>35.4 ± 15.5</td>
<td>0.94 (0.65, 1.36)</td>
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<tr>
<td>CL/F (L/h)</td>
<td>834 ± 928</td>
<td>722 ± 440</td>
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<tr>
<td>t½</td>
<td>3.75 ± 0.66</td>
<td>3.57 ± 1.11</td>
<td>0.92 (0.5, 1.13)</td>
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<tr>
<td>tmax (h)</td>
<td>1.0 ± 0.5</td>
<td>1.1 ± 0.4</td>
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<tr>
<td>Cmax (ug/L)</td>
<td>18.8 ± 12.2</td>
<td>14.2 ± 6.7</td>
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<tr>
<td><strong>R-416</strong></td>
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<tr>
<td>AUC0-24h</td>
<td>3.6 ± 1.1</td>
<td>3.3 ± 1.3</td>
<td>0.84 (0.40, 1.74)</td>
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<tr>
<td><strong>Pravastatin lactone</strong></td>
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</tr>
<tr>
<td>AUC0-24h</td>
<td>26.5 ± 17.4</td>
<td>28.7 ± 23.1</td>
<td>0.89 (0.68, 1.15)</td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration-time curve; Cmax, peak plasma concentration; tmax, time of peak plasma concentration; t1/2, terminal half-life. Results at baseline and after garlic intake are given as mean ± SD. The last column gives the point estimate and the 95% CIs of the geometric mean ratio of the results observed after garlic intake and at baseline.
Table 2: Saquinavir kinetics before and after garlic extract intake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Garlic</th>
<th>Geometric mean ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saquinavir</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0-8h (ug . h/L)</td>
<td>1888 ± 578</td>
<td>1698 ± 882</td>
<td>0.85 (0.66, 1.09)</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>73.6 ± 38.7</td>
<td>91.1 ± 57.7</td>
<td></td>
</tr>
<tr>
<td>t½</td>
<td>1.33 ± 0.22</td>
<td>1.67 ± 0.71</td>
<td>1.20 (0.94, 1.52)</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>3.1 ± 0.6</td>
<td>2.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Cmax (ug/L)</td>
<td>729 ± 231</td>
<td>659 ± 287</td>
<td></td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration-time curve; Cmax, peak plasma concentration; tmax, time of peak plasma concentration; t½, terminal half –life.

Results at baseline and after garlic intake are given as mean ± SD. The last column gives the point estimate and the 95% CIs of the geometric mean ratio of the results observed after garlic intake and at baseline.
Figure 1:

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Garlic</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Here is a table summarizing the results:

<table>
<thead>
<tr>
<th></th>
<th>PGP</th>
<th>Villin</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>positive control</td>
<td>positive control</td>
<td>positive control</td>
</tr>
<tr>
<td>PGP</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
<tr>
<td>Villin</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
</tbody>
</table>
Figure 2:
Figure 3:
Figure 4:

- AUC pravastatin [ug.h/l]
- Pravastatin and lactone [ug/l]
- Time (h)
- R-416 [ug/l]
- Time (h)

Graph showing the concentration of pravastatin and lactone over time with AUC calculations.
Figure 5:

[Graph showing the concentration of saquinavir over time for Control and Garlic conditions, with AUC calculations.]